Vegetable Science (2019) 46 (1&2) : 1-16

Root knot nematodes menace in vegetable crops and their management in India: A Review

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Received: May 2018 / Accepted: June 2019

Abstract

Vegetables are chief constituents of Indian diet and play a pivotal role in ensuring nutritional and livelihood security to the increasing Indian population. However, vegetable production is seriously hampered by several biotic stresses, which pose huge challenge to meet future demands of Indian population. Among several biotic stresses, root knot nematodes (RKN) are major threat to vegetable production. Root knot nematodes (*Meloidogyne* spp.) are obligate, sedentary root endoparasite and belong to one of the most economically important groups of plant-parasitic nematode genera. Besides the direct damage, root knot nematodes act as predisposing agent for the entry of soil borne fungal and bacterial pathogens and aggravate the problem still further leading to development of disease complexes. Although the management of this nematode pest is largely done using chemical nematicides however, phase out of these nematicides due to its toxic hazard on human beings as well as environment, the problem of root knot nematodes intensified and become a major stumbling block for successful cultivation of vegetable crops in open field as well as protected cultivation. Thus, in this review an attempt has been made to comprehend the multitude of cultural, physical, biological, chemical and genetics-based methods for the management of root knot nematodes in vegetable crops with special reference to India.

Key words: Vegetables, root knot nematode, economic damage, races, parasitism, management, integrated approach.

Introduction

Root knot nematodes of the genus *Meloidogyne* are one of the most economically important groups of plant-parasitic nematode genera. They are obligate, sedentary root endoparasite infecting more than 3000 plant species (Abad et al. 2003). Considering to their economic importance on a worldwide basis, they rank high on the list of animate pathogens affecting world food production and allied sectors (Sasser 1977). They distributed worldwide over a wide range of geographical conditions, more prevalent in tropical and sub-tropical climatic zones and cause huge economic loss in a multitude of agriculture and horticulture crops including vegetables (Sikora and Fernandez 2005). In vegetable crops, it is generally admitted that *Meloidogyne incognita* Kofoid and White, *M. javanica* Treub, *M. arenaria* Neal and *M. hapla* Chitwood are the four major species reported to cause huge economic damage among described species in the genus. The former three species found in tropical and subtropical regions, are also found to present temperate areas particularly under protected cultivation. The later species *M. hapla* is typically present in temperate areas and at higher altitude in the tropics (Hunt and Handoo 2009). In India, *Meloidogyne incognita* is the most frequently observed species among the genus followed by *M. javanica* and *M. arenaria* in vegetable cultivation. Moreover, *M. hapla* is known to infest vegetables crops grown in temperate hilly regions. In addition, they not only reduce crop yield but also affect the quality of vegetables (Seid et al. 2015).

Economic importance and extent of damage

Globally, an average of 10% annual yield loss is recorded in vegetables due to root knot nematodes. However, much higher percent losses have been recorded depending on nematode species, locality, crop species and soil population level (Collange et al. 2011). Sikora and Fernandez (2005) recorded over 30% yield losses in tomato, eggplant and melons. In India, the annual monetary loss was estimated to the tune of 5131.80 million rupees due to infestation of root knot nematodes in major vegetable crops (Jain et al. 2007) (Table 1). Besides the direct injury, root knot nematodes act as

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predisposing agent for the entry of soil borne fungal and bacterial pathogens and aggravate the problem still further leading to development of disease complexes and causing severe yield losses between 40-70% in vegetable crops growing various parts of the country (Rao et al. 2015a). In addition, crops such as tomato, chilli, gherkins, okra, muskmelon, watermelon and flower crops such as carnations, roses, gerbera and anthuriums are being grown under protected cultivation are severely infested by root knot nematode species (*M. incognita, M. javanica* and reniform nematode (*Rotylenchulus reniformis*) and cause huge crop loss (up to 80%) in selected crops (Rao et al. 2015).

Table 1: Estimated annual yield and monetary loss in different vegetable crops due to infestation of root knot nematode

Sl. No.	Vegetable crops	Yield loss (%)	Monetary loss (Million rupees)
1	Tomato	27.21	2204.00
2	Brinjal	16.67	1400.30
3	Chilli	12.85	210.00
4	Okra	14.10	480.00
5	Cucurbits	18.20	547.50
6	Carrot	10	290.00
		Total	5131.80

Races of root knot nematodes and their distribution

Root knot nematodes races are known to occur in four prominent species of the root knot nematodes such as *M. incognita* and *M. javanica, M. arenaria* and *M. hapla*. Sasser (1952) first reports the host range variations in four major *Meloidogyne* species. Taylor and Sasser, (1978) used the term 'race' to *Meloidogyne* populations. In India, occurrence of 4 races (race 1, 2, 3 and 4) in *M. incognita* (Sharma and Gill 1992; Khan 1997), 3 races (race 1, 2 (pepper race) and 3 (groundnut race) (Sharma et al. 1995; Khan et al. 2003) of *M. javanica* and 1 race (race 2) of *M. arenaria* (Khan et al. 1993; Kumar et al. 2008) have been reported from the different parts of the country. Recently Khan et al. (2014) first time recorded the occurrence of two new races in *M. incognita* (race 5 and 6) and three new races in *M. javanica* from India. In *M. incognita*, out of four races, race 1 is widely prevalent in India and race 2, 3 and 4 are reported to present in selected parts of the country. Similarly, the races of *M. javanica* and *M. arenaria* distribution in different parts the country is presented in table 2.

Parasitism and symptom expression

Owing to their parasitism, second stage (J_2) infective juveniles moves freely in soil in search of suitable host

Table 2: Status and distribution of root knot nematodes races (Khan et al. 2014)

Meloidogyne species	Races	Distribution in different States/Union Territory
M. incognita	1	India
	2	Andhra Pradesh, Assam, Bihar, Gujarat, Haryana, Himachal Pradesh, Karnataka, Kerala, Maharashtra, Mizoram, Odisha, Punjab, Tamil Nadu, Uttar Pradesh, West Bengal
	3	Assam, Gujarat, Haryana, Karnataka, Maharashtra, Puducheri, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal
	4	Haryana, Himachal Pradesh, Uttar Pradesh
	5	Haryana, Maharashtra, Tamil Nadu, Tripura
	6	Manipur
M. javanica	1	Haryana
	2	Uttar Pradesh, West Bengal
	3	Andhra Pradesh
	4	Andaman and Nicobar Islands, Gujarat
	5	Uttar Pradesh, West Bengal
	6	Haryana
M. arenaria	2	Haryana, Uttar Pradesh

and then penetrate to root tips by injecting hydrolytic enzymes secreted by oesophageal glands into plant cells through its stylet and migrate intercellularly until it reaches the differentiating vascular cylinder (Dasgupta and Gaur 1986; Dutta et al. 2015a). This migration proceeds for short distance and further J_2 become sessile in the zone of differentiation (cortical tissue). Generally, head of J_2 embedded in vascular tissue and rest of the body in the cortex which is parallel to longitudinal axis of the roots and initiate feeding on xylem and phloem cells and induce specialized multinucleate feeding cell or giant cells in the phloem or adjacent parenchyma. Multinucleated giant cells are feeding sites normally formed due to repeated endomitosis without cytokinesis and adjacent cells surrounding the giant cells are proliferated through hyperplasia giving rise to numerous knots or galls on root system. These cells are highly specialized cellular adaptations serve as the permanent food source for nematode development and reproduction (Davis et al. 2008). Second stage juveniles continuously feed optimally for 2-3 weeks and under favourable conditions, it undergo series of moults in quick succession into third and fourth juvenile stages in short span of time typically 4-6 days (Moens et al. 2009). Third and fourth juvenile stages are non-feeding stages due to lack of functional stylet. Then, majority of fourth stage juveniles develop into pyriform sedentary females and rest become vermiform males. There is no evidence of feeding of males on plant cells. The females lay eggs into gelatinous matrix to protect from environmental extremes and predation. Under favourable condition, second stage juvenile hatch out from egg by proceeding first stage moult in egg itself. The total duration of life cycle varies with species, host and environmental conditions, but under optimum condition life cycle completes in 3 to 4 weeks (Dasgupta and Gaur 1986).

Root knot nematodes typically detected first in localized areas within a vegetable field, nursery or kitchen garden. Affected plants usually express formation of knots or galls on roots. Galls around the feeding site in vascular tissue, affect the upward translocation of water and nutrient in the root resulting in qualitative and quantitative crop loss (Moens et al. 2009). However, size of the root gall varies with the host plants and nematode species involved. The galls produced on cucurbitaceous crops are much larger than the ones produced on chilli. In addition, other typical symptoms such as forking of tap roots in carrot or beet and pimple-like tubercles on tubers of potato are also produced. Root knot nematode infected plant express above ground symptoms like mineral deficiency or drought stress symptoms such as chlorosis, yellowing of leaves, wilting and stunted growth.

Influence of soil factors on nematode biology

In soil environment, abiotic and biotic factors largely govern the nematode behavior. Temperature and moisture are the main driving abiotic factors in soil ecosystem; but, other factors of soil influence the population behavior of nematodes and their effects on the host plant (Gaur 2006). Temperature, moisture and soil type are noted as the most important soil abiotic factors influencing the distribution, life cycle, survival and pathogenicity of root knot nematodes. Generally, three prominent species of root knot nematode *M. incognita, M. javanica* and *M. arenaria* generally need relatively higher temperature for multiplication and survival than *M. hapla*. Hence, former three species were dominant in tropics and subtropics. However, later species distribution limited to temperate and sub temperate regions. At geographical location, the temperature determines the population behaviour of root knot nematodes. In northern plains of India, peak populations of root knot nematodes are generally observed during September/October months and March/ April months (Dasgupta and Gaur 1986). The duration of life cycle and number of generations of root knot nematode species highly depends on temperature and host species. The root knot nematode species like *M. incognita* and *M javanica* completes life cycle in 21-25 days at 26-27°C While, at 14-16°C the life cycle extends up to 50-60 days or even 80 days also. The optimum temperature lies between 25- 30°C is favorable for *M. incognita* and *M. javanica* (Mohiddin and Khan, 2014) and average annual temperatures of 18° to 27°C were suitable for *M. arenaria* (Taylor et al. 1982). However, the temperature levels between 25-28°C and light textured soils are highly suitable for the juvenile movement, invasion, rapid multiplication and gall formation (Mohiddin and Khan 2014). Cold climate species, *M. hapla* restricted to sites with average annual temperature 24-27° C and significantly distributed in warmer latitudes where rainfall well distributed throughout the year and places of cold winters and warm wet summers. In India, Anita and Selvaraj (2011) reported that, *M. hapla* able to complete its life cycle within 26 days at 15-22°C on carrot crop in Nilgiri hills.

Nematodes require thin film of water for their survival and locomotion (Wallace 1969). Moisture level below (40-60%) field capacity is optimum for the activity of root knot nematode species, however they adapted to survive under low or high moisture levels. At higher moisture levels with low oxygen levels second stage juveniles undergo anoxybiosis and hatching is inhibited. However, in dry condition the second stage juveniles remain active if soil air has 100% relative humidity and hatching inhibited due to high osmotic pressure in egg sacs. Therefore, mass hatching is occurring when the soil is irrigated after dry spell. They are generally more abundant in sandy and sandy loam soils with 50% or more sand. The movement of infective juveniles and root penetration become easy and more, when the ratio of particle diameter to length of juvenile is 1:3 (Wallace 1969). Nevertheless, root knot nematodes inhabit a wide variety of soils, however their damage level relatively less in clay soil. Generally infective juveniles more abundant in the 20 cm soil layer at field condition, but vertical distribution may be influenced by temperature and moisture changes (Dasgupta and Gaur 1986).

Management

High reproductive potential, polyphagous nature and unique survival mechanism made management of root knot nematodes more difficult under intensive vegetable cultivation. Once the root knot nematodes are established in field, it is virtually impossible to eradicate. However, eradicating nematodes is neither economically nor ecologically sounds unless there is regulatory requirement for total control of nematodes. Hence the concept of "living with nematodes" (Tyler 1933) has been strengthened and nematologists have made sustained efforts to develop ideal approaches to manage this nematode pest. Thus, in this review an effort has been made to identify and analyze a multitude of cultural, physical, biological, chemical and genetics-based methods for the management of root knot nematodes in vegetable crops with special reference to India. Even though, the literature on management of root knot nematode is abundant, we carefully elaborated appropriate, cost effective strategies for the management of root knot nematodes in vegetable crops. Accordingly, first we covered the effect of individual approach and their mode of action and in final part; we outlined the integrated approach.

Cultural methods: Cultural practices have remarkable significance in the management of nematodes as well as other pests and diseases. In soil environment abiotic and biotic factors largely govern the nematode behavior. Soil temperature and soil moisture, chemical and physical composition of soil, antagonistic flora and fauna are extremely influence the nematode behavior and their effects on hosts. Therefore, for effective management it is imperative to disturb the harmonious relationship between nematode and host plant through altering soil ecosystem by strategic approaches (Gaur 2006). Hence, major cultural practices such as sanitation, deep summer ploughing, crop rotation, utilization of trap crops, cover crops and antagonistic crops, application of organic amendments, weed management and destruction of crop residues are play a major role in nematode management.

1. Sanitation: Sanitation is the solitary principle to prevent new-area infestations and to avoid secondary spreading of root knot nematodes in vegetable field. Generally, root-knot nematodes are easily spread through vegetative propagules (Collange et al. 2011; Rao et al. 2015b). Hence as preventive measure obtains nematode free planting material from reliable source. In general, weeds act as alternate hosts for root knot nematodes. In vegetable cropping system, weeds such as *Chenopodium album*, *Solanum nigrum*, *Tithonia rotundifolia* and other unknown weeds are known to act as excellent hosts for the perpetuation of root knot nematodes (Khan et al. 2014). Thus, timely removal and destruction of weeds generally helps to minimize the inoculum level under field condition. In addition, they can survive in crop residues. These crop residues enhance the rate of survival of nematodes by slowing the rate of desiccation and providing mechanical protection during adverse conditions. Therefore, removal and destruction of crop residues after crop maturity helps in reducing inoculum densities to succeeding crop. At farm level, agricultural machineries and tools should be cleaned to avoid spreading of nematodes (Sethi and Gaur 1986).

2 Summer ploughing: Two or three deep summer ploughing in the hot summer months expose the nematodes and infected tissue to solar heat and dehydration. This practice has been found effective to manage root-knot nematodes (Jain and Bhatti, 1987). In brinjal, fallowing and summer ploughing during hot summer months significantly reduce the *M. incognita* (Singh, 2013). According to Jain and Gupta (1990) normal (10 cm) and deep ploughing (20 cm) during the month of June followed by a fallow period of about two months recorded significant reduction of root-knot nematode population ranging between 78.2 to 92.3%, respectively at the time of transplanting of tomato. Similarly, in okra, normal and deep ploughing reduced *M. javanica* population 79.2 and 76.3% respectively. The efficiency of summer ploughing can be improved by soil solarization, which helps in trapping and retaining more heat under polyethylene mulching than direct exposure alone (Gaur and Perry 1991).

3 Crop rotation and Cropping sequence: Rotation of crops is widely used effective cultural practice to reduce root knot nematode populations in the soil. The crop rotation with graminaceous poor hosts and certain antagonistic crops for one or two years have been found effective to bring down inoculum level of root knot nematodes (Sundresh and Setty 1977; Patel et al. 1979). Considering to certain cropping sequences, non-preferred hosts like sesame, mustard, wheat, maize, etc. have been found to suppress the root knot nematode population (Haque and Gaur 1985; Siddiqui and Saxena 1987). The key principal involved in this method is starvation and interruption in their life cycle. Rotation of non-host crops such as mustard, garlic, onion and cereals at least for 2 to 3 years in a suitable cropping system helps in minimizing inoculum level of root knot nematodes (Khan et al. 2010). Cropping sequence in vegetable based cropping systems plays a key role in nematode management. However, sometimes vegetable based cropping sequence predominantly increases nematode damage potential in vegetable crops. Chandra and Khan (2011) found that the sequence of okra-brinjal-okra stimulated root knot nematode population under field condition. In contrast to this, cropping sequences such as okra-cowpea-cabbage and okra-cucumber-mustard were found to be effective to suppress the *M. incognita* population in field condition. Similarly, Siddiqui and Alam (2001) evidenced that, the cropping sequences of sorghum-wheat-horse gram-turnip and fallow-cauliflower-sorghum-coriander were found to have a maximum suppressive effect on root knot nematode *M. incognita* and reniform nematode *R. reniformis*.

4. Trap crops, antagonistic crops and cover crops: Trap crops, cover crops and antagonistic crops are normally termed as nematode suppressive crops. These crops inhibit or reduce the nematode population by their planting or incorporation. Trap crops are highly susceptible crops grown in root knot nematode infested fields and allowed to grow over a time period to invade and develop but do not support for complete its life cycle. *Crotalaria spectabilis* is the most commonly used as trap crop against root knot nematodes. However, success of this practice is completely depending upon timing and total crop destruction (Viaene et al. 2006; Gaur 2006). The crops which have nematode antagonistic properties majorly from its root exudates can be utilized as rotation crop, cover crop or as inter crop with susceptible crop. Crops like marigold (African marigold, French marigold, South American marigold), mustard, sesame, asparagus (*Asparagus officinalis*) are known to have nematode suppressive activity by releasing nematotoxic compounds (Table 3) through root exudates (Gaur 1975; Haque and Gaur 1985) of these, marigold is the most studied crop which have ability to suppress nematode activity by releasing polythienyls toxic compounds. Marigold intercropping with tomato, okra, brinjal in different season's significantly reduced root knot nematode incidence by reducing soil nematode population 36.2, 53.5 and 72.9% respectively and percent reduction of root galls 45.4, 40.1 and 86.2% respectively, over control after 90 days of transplanting/ sowing (Umashankar et al. 2005).

Cover crops are extensively used for management of plant parasitic nematodes. In general, a cover crop is planted or incorporated between the cultivation of an annual cash crop. The principle of cover crops is to supply organic amendments, improve soil structure, cycling of nutrients, protect from soil erosion and suppress weeds, insects, nematodes, and other plant pathogens (Pankaj et al. 2006). Cover crops are generally exploited to manage nematodes, because nematodes move only very short distances on their own and cannot migrate to another field if a cover crop is not a host to the nematodes or some population may starve, which helps to reduce initial population density to next crop. In addition, incorporating cover crops as green manures suppress the nematode population by releasing nematotoxic compounds after decomposition (Table 3) (Chitwood and David 2002). In general, crops such as *Crotalaria,* castor bean, velvet bean, jack bean, sorghum-sudan, castor, grasses and cereals have been successfully utilized as cover crops for root knot nematode management (Hackney and Dickerson 1975; Viaene et al. 2006).

Table 3. Nematicidal compounds in nematode suppressive crops

Sl. No.	Nematode suppressive crops	Nematicidal compounds
1.	Marigold	Polythienyls-*Alpha-terthienyl*
2.	*Crotalaria* spp.	Monocrotoline, Pyrrolizidine
3.	*Secale cereale* (Rye)	Butyric acid and Hydroxamic acid
4.	Sudan grass	Cyanoglycoside dhurrin
5.	Castor	Ricin
6.	Velvet bean	1-tricontanol, triacontanyl tetracosanate
7.	Brassicas (Rape seed and Mustard)	Isothiocyanates
8.	Sesame (*Sesamum indicum*)	Acetic acid

5 Organic amendments: Use of organic amendments is a traditional agricultural practice in Indian farming to enhance soil fertility, soil physical condition, recycling of nutrients and soil biological activity. However, several studies evidenced that; organic amendments also can be utilized for the management root knot nematodes including other plant parasitic nematodes (Alam 1976; Akhtar et al. 1990; Addabdo 1995). Generally, organic amendments are polysemic (Collange et al. 2011) includes organic manures (animal and poultry), plant parts and their extracts, plant products, industrial wastes, green manures from cover crops, vermicomposts, etc. The three major biological processes in organic amendments which helps in suppression nematode population are (a) stimulate intense microbial activities including nematode antagonists, predators and parasites in the soil during decomposition. (b) After decomposition, the released specific compounds may be nematicidal (c) Enhance the soil capacity to withhold nutrients, which improves plant vigour and plant tolerance to nematodes (Bridge 1996; Akhtar and Mahmood 1996; Oka 2010). In this endeavor, some relevant studies on different forms of organic amendments for the management of root knot nematodes in vegetable crops are examined and documented in this review as follows.

a. Green manuring: Incorporation of chopped Brassica (*Brassica juncea*, *B. napus, B. rapa*) green manures into the soil also limits the reproduction of nematodes. After decomposition, they release volatile compound like isothiocyanates produced from glucosinolates, which are toxic to plant parasitic nematodes including root knot nematodes. This process generally termed as bio-fumigation (Ploeg, 2007). Randhawa and Sharma (2008) revealed that, application of *Brassica rapa* (*var.* TL150) as soil amendments reduced 52.17% of root galls and enhanced seedling height and weight 26.2 and 34.5% respectively, in tomato plants. Seed as well as root dip treatment of *Calotropis gigantia* leaf extracts significantly reduced root knot nematode population after 45 days of transplanting and also at the time of harvest in tomato crop (Saravanapriya and Sivakumar 2005). Ahmad et al. (2010) demonstrated that, leaf extracts of *Lantana camara* was found to be highly nematostatic, where juveniles were completely paralyzed after 12 hour of exposure and 96% of juvenile's mortality was observed at 48 HAE. Under *in vivo* condition, addition of freeze-dried extract around the rhizosphere of eggplants significantly reduced the juvenile's penetration. In brinjal, application of sunhemp (*Crotolaria juncea*) as amendment reduced the nematode population in soil and roots (Patel and Dhillon 2017)

b. Leaf extracts and products: Rather et al. (2008) found that, pot application of 100g of chopped leaves of neem, Persian lilac and marigold considerably reduced the incidence of root-knot nematode in tomato with enhancing plant growth in tomato. Similarly, the application of madar (*Calotropis procera*) and neem (*Azadirachta indica*) chopped leaves considerably reduced the nematode population in soil with lesser root knot index under pot experiment (Singh and Patel 2015). Further Asif et al. (2017) revealed that the application of chopped leaves of *Mexican poppy*, *Trailing eclipta* and Wild eggplant in combination with wild spinach powder found to be effective for suppression of root knot nematode incidence in tomato.

c. Oil cakes and organic manures: Solid press cakes or oil cakes have been widely used and recommended for suppression of nematode population in soil. Goswami and Meshram, (1991) found that, application of mustard and karanja cake as soil amendments reduced the juvenile's penetration on tomato roots. They noted that, cakes efficacy as similar to carbofuran treatment. Similarly, several studies evidenced that, neem cake (*Azadirachta indica*) even at low dosages (1-2 t/ha) can effectively reduce the root-knot nematode incidence in vegetable crops (Akhtar and Malik 2000; Devi and Das 2016). Several studies demonstrated that, Farm yard manure (FYM), Vermicompost and Poultry manure can suppress nematode population (Kumar et al. 2011; Singh et al. 2014; Kankam et al. 2014). However, due to slow release of nematicidal compounds may result in their concentrations being too low to be effective on nematodes (Akhtar and Mahmood 1997). Nevertheless, if critical products are maintained for a long time, the organic amendment or compost may stimulate the increased activity of biological antagonists of nematodes (McSorley and Gallaher 1995). However, the efficacy of organic amendments is merely depending on C: N ratio and time of decomposition. In addition, C: N ratio of organic matter plays a prime role in microbial decomposition and nitrogen uptake by higher plants. Even, some studies evidenced that, potential of nematode management is directly related to its nitrogen content of organic amendment (Mian and Rodríguez-Kábana 1982). In general, studies are proved that, low carbon: nitrogen (C/N) ratios in organic amendments (oilcakes, animal manures, and green manures) exhibit high nematicidal activity (Lazarovits et al. 2001; Oka, 2010). This phenomenon is attributed to the release of ammonia during the decomposition of the amendment in soil (Rodriguez-Kabana 1986; Oka et al. 1993). In contrast, low C: N ratio may exhibit phytotoxicity on plants. According to Rodriguez-Kabana et al. (1987) C: N ratio of organic amendment is between 12-20 could be beneficial to exhibit nematicidal activity and to avoid phytotoxicity.

Physical methods

Physical methods are one of the important management methods normally utilized either alone or in combination with cultural and chemical management methods. The physical methods are relying on use of heat as nematodes have different maximum and minimum temperature thresholds for its survival, activity, infection and growth. Hence, the key abiotic factor, temperature can be exploited for management of nematodes. Among important physical methods, soil solarization is effectively used for the management of root knot nematodes infesting vegetables.

Soil solarization is a method of heating moist soil by covering it with transparent plastic sheets to trap solar radiation during hottest period or summer season of the year. The principles of soil solarization was given in detail by Katan (1980) which are (a) accumulation of heat due to transmission of short wave solar radiation and prevents loss of long wave radiation in solarized soil, (b) soil moisture helps in solarization process by conducting heat energy, (c) increase in temperature due to greenhouse effect, (d) increase in microbial and physico-chemical reactions in the soil resulting in to accumulations of gases, some being toxic pathogens and others acting as a nutrient source or induce resistance to subsequent crop and (e) prolonged exposure to higher temperature resulting in increased mortality of nematodes and also making them susceptible to nematode antagonists. According to Katan et al. (1983) the period of solarization should be adequate to transmit heat into deeper layers of soil. The solarization period is ranging between 2 to 9 weeks have been reported to be effective for nematode suppression. Nevertheless, soil properties (texture, structure, chemical composition, and moisture content), quantity and availability of solar radiation and the thermal sensitivity of target organisms and cropping sequences determines the optimum period of soil solarization (Jain 2006). However, Gaur and Dhingra (1991) revealed that, 4 to 6 weeks of solarization in the period of mid-summer have been found effective under tropical and subtropical conditions to reduce nematode incidence. In addition, nature of polyethylene sheets and their size influence on soil solarization. The use of transparent sheets produces better results than black sheets, since transparent sheets transmit most of the incident radiation to the soil (Mehrer and Katan, 1980). The thickness of plastic sheets between 50 and 100 µm is optimum for soil solarization (Jain 2006). The effect of soil solarization on root knot nematodes infecting vegetables was proved by many studies for example, Jain and Gupta (1996) revealed that, solarization during May to June months reduced 78% of *M. javanica* soil nematode population. In nursery, soil solarization with clear and black LLDPE (Linear Low-Density Polyethylene) mulch reduced 92.5 and 87.4% of soil population of *M. javanica* respectively (Jain and Gupta 1997). Soil solarization through 100 gauze (25 µm) LLDPE clear plastic film for 15 days during May month reduced the root knot disease and weeds by 66% and 93% respectively (Walia et al. 2016).

Biological control

Globally, numerous microorganisms have been reported as nematode antagonists (Poinar and Jansson 1988) and noted to be widely distributed in cultivated soils of different climatic and environmental conditions (Sayre and Starr 1988). Normally, nematode antagonists belong to fungi and bacteria. These antagonists feed or parasitize the nematodes or release secondary metabolites with nematicidal activity. Therefore, exploitation of nematode antagonists for the suppression of root knot nematodes is one of the promising methods of nematode control. The importance of nematode antagonists to manage plant parasitic nematodes was stimulated with the discovery of predatory nematodes (Cobb 1917), nematode-trapping fungi (Linford and Yap 1939), and nematode parasite *Pasteuria penetrans* (Thorne 1940). Nematode suppressive soils are made by inoculation with effective antagonists at higher concentration to attain immediate control normally termed as inundation strategy as well as inoculation strategy in which long-term effects are achieved through colonization of nematode antagonists in soil. Different groups of nematode antagonists are discussed as follows.

1 Fungal antagonists: Nematode antagonistic fungal bio-agents generally belong to soil borne fungi group. These fungal bio-agents can be grouped into nematode-trapping or predacious fungi, egg and cysts parasitic fungi, endoparasitic fungi and fungi that produce toxic metabolites against nematodes.

a. Nematode trapping fungi: *Arthrobotrys* spp. and *Monacrosporium* spp. are the two fungal antagonists which trap nematodes in constricting rings and adhesive nets respectively (Thakur and Devi, 2007). Their predation mechanism involves the association between a lectin secreted by the fungus and a carbohydrate secreted by the nematode cuticle. Some reports revealed that, they predate the root-knot nematodes *M. incognita* (Kumar and Singh 2006; Thakur and Devi 2007), *M. javanica* (Khan et al. 2006) infecting vegetable crops. However, in contrast their predation is specific to certain nematode species and restricted availability of these antagonists in soil limits their potential use.

b. Egg parasites: A considerable range of fungi (*Paecilomyces lilacinus*, *Pochonia chlamydosporia*, *Dactylella oviparasitica*, *Nematophthora gynophila* and *Cladosporium oxysporum*) have been reported as egg as well as female parasites of root knot nematodes (Khan and Goswami, 2001). Among, *Paecilomyces lilacinus* is the fungal bioagent commercially exploited for the control of plant parasitic nematodes. The sedentary stages such as egg mass, obese female of root knot nematodes are vulnerable to fungal colonization or parasitization. The infection process initiates with the growth of fungal hyphae in the gelatinous matrix and penetrates individual hyphae through egg cuticle and then eggs are engulfed with mycelial network. *P. lilacinus* proved its high antinemic activity by reducing incidence of root-knot nematode species, *M. javanica* and *M. incognita* in tomato, brinjal and other vegetable crops (Goswami and Mittal 2004; Goswami *et al.* 2006; Haseeb and Kumar 2006; Ganaie and Khan 2010). In India *P. lilacinus* commercially available with different trade names and effectively utilized for the root knot nematode management in vegetable crops (Khan et al. 2014). Similarly, another egg parasitic fungus *P. chlamydosporia* proved its potential egg parasitism in root knot nematode *M. incognita.* Chaya and Rao (2012) revealed that, this fungus considerably reduced nematode population in okra.

c. Toxin producing fungi: Most prominently, the filamentous fungi *Trichoderma* spp. (*Trichoderma viride, T. harzianum*), strains commercially used for the management of root knot nematodes infecting vegetable crops (Rao et al. 1998; Goswami and Mittal 2004; Haseeb and Khan 2012). The mode of action of *Trichoderma* spp. involves two mechanisms a) direct parasitism on eggs through increasing extracellular chitinase activity; b) induce systemic resistance in plants (Sahebani and Hadavi 2008).

2 Antagonistic Bacteria:

a. Spore forming bacteria: *Pasteuria penetrans* (Thorne) Sayre and Starris the most studied bacterial antagonists of plant parasitic nematodes. *Pasteuria penetrans* is gram positive endospore-forming, obligate parasitic bacteria widely distributed in agricultural soils throughout the world (Sayre and Starr 1988; Hewlett et al. 1994). Many studies proved their potentiality against root knot nematodes infecting different vegetable crops (Walia and Dalal 1994; Swarnakumari and Sivakumar 2012; Swarnakumari 2017). The parasitism is mediated by attachment of adhesive endospores on second-stage juvenile's cuticle and their proliferation inside nematode body by utilizing resources for its growth by synchronizing its life cycle with nematode development and causing sterility in females by disrupting nematode reproductive system (Vagelas 2012). However, the efficacy of this bacterium is highly influenced by soil porosity, irrigation practices, soil structure, soil texture and number of endospores present in soil. In addition, having constraint in large scale mass production due to obligate parasitism limits its commercial utilization.

b. Plant growth promoting rhizobacteria (PGPR): Plant growth promoting rhizobacteria are potentially exploited as nematode antagonists against plant parasitic nematodes including root knot nematodes. Generally, plant growth promoting rhizobacteria are most abundant in rhizosphere region of plant. Their multiple mode of action such as competition for nutrients, antibiosis, plant growth promotion and induction of systemic resistance in plants against nematodes proved them as potential nematode antagonists. Among known plant growth promoting rhizobacteria, *Pseudomonas fluorescens* and *Bacillus* spp. are the most studied bacterial antagonists against root knot nematodes. Many studies proved the efficacy of *P. fluorescens* and *Bacillus subtilis* biocontrol agent against root-knot nematodes infecting vegetable crops (Krishnaveni and Subramanian 2004; Haseeb and Kumar 2006; Stalin et al. 2007; Thiyagarajan and Kuppuswamy 2014; Rao et al. 2017a, 2017b).

3 Enrichment of biocontrol agents to improve their efficacy: The biocontrol efficacy of an antagonist is likely to be affected by several biotic and abiotic factors. Therefore, efforts were predominantly made to enhance the biocontrol potential of nematode antagonists by using organic amendments (Muller and Gooch 1982). Several studies revealed that, organic amendments act as substrate material for nematode antagonists to provide suitable environment for their growth and development. Many studies shown that, nematode antagonists enriched with organic amendments (Farm yard manure, vermicompost, neem cake, mustard cake and karanja cake) exhibited significant control of root knot nematodes in different vegetable crops (Goswami and Meshram 1991; Singh 2013; Singh et al. 2014; Parihar et al. 2015; Rao et al. 2017b). Formulations of different biocontrol agents commercially available in India for the management of root knot nematodes infecting vegetable crops are enlisted in table 4.

Chemical control

Nematologists made continuous efforts to develop chemical approaches to manage nematode pest by discovering nematicidal properties of three chemicals namely methyl bromide, D-D mixture, and ethylene dibromide in the decade between 1940 and 1950 and subsequently for other nematicides. However, phase out of these fumigant nematicides due to health as well as environmental hazards (Abawi and Widmer 2000), the problem of plant parasitic nematodes majorly root knot nematodes are still intensified. Presently, only few chemicals are registered for nematode control in vegetables such as Dazomet in tomato nursery, Carbofuran 3% CG for cabbage and brinjal and Flupyram 34.48% w/w SC in tomato (http://ppqs.gov.in/divisions/cib-rc/major-uses-of-pesticides).

Table 4. Commercial formulations of biocontrol agents for the management of root knot nematodes infecting vegetable crops in India.

Bioagents	Trade names	Manufacturer	References
Pseudomonas fluorescens 1% W.P. *Trichoderma harzianum* 1% W.P. *Trichoderma viride* 1.5% W.P. *Paecilomyces lilacinus* 1% W.P. *Pochonia chlamydosporia* 1% W. P.		ICAR-Indian Institute of Horticulture Research, Bengaluru	https://www.iihr.res.in/ Rao *et al.,* 2015
Paecilomyces lilacinus 1% W.P.	PAECILO®	PAECILO® Agri Life Bio solutions for soils & Crops, Hyderabad	http://www.agrilife.in
Paecilomyces lilacinus 1% W.P.	MYSIS®	MYSIS® Varsha BioScience and Technology, Hyderabad	http://www.varshabioscience.com
Paecilomyces lilacinus 1% W.P.	BIONICONEMA®	BIONICONEMA® Nico Orgo Manure, Gujarat	http://www.neemnico.com/
Pseudomonas fluorescens 1% W.P.	POWER ALL	POWER ALL® Nico Orgo Manure, Gujarat	http://www.neemnico.com/
Paecilomyces lilacinus 1% W.P.	BioAce®	Indore Biotech Inputs & Research (P) Ltd, Indore	http://www.indobioagri.in
Paecilomyces lilacinus (Wettable Powder and Aqueous Suspension)	Nemator®	Biotech International, New Delhi	https://www.biotech-int.com
Paecilomyces lilacinus 1% W.P.	Nemastin®	Kans Biosys, Maharashtra	http://kanbiosys.com

Genetics based methods

Genetics based methods of nematode control includes use of resistant/ tolerant varieties developed by classical plant breeding and genetic engineering approach. Identifying the source of resistance and its utilization is the best option for nematode management because resistant varieties compatible with other management methods. In recent decade, genetic engineering a new approach began in the field of nematology which provides a strategy to design effective, durable resistant crops against economically important plant parasitic nematodes. In recent years, many studies were began in the country on application of biotechnological approach such as RNA interference and proteinase inhibitors to combat the root knot nematodes are briefly discussed below.

1 Host plant resistance: Breeding for nematode-resistant cultivars are of much significance as effective and environmentally safe alternative to chemical nematicides (Reddy *et al.* 2016). In this view, evaluation of germplasms/genotypes or wild relatives of different vegetable crops are prerequisite for identifying resistant sources to edifice breeding programme. Roberts (1995) reviewed that, several wild plant species have natural source of resistance against root knot nematodes, *Meloidogyne* spp. In this context, studies were initiated to identify sources of resistance, for example Williamson et al. (1998) and Milligan et al. (1998) found resistance *Mi* gene from the wild tomato species *Solanum peruvianum* which conferred resistance to three economic species of root knot nematodes, *Meloidogyne incognita, M. javanica and M. arenaria,* Similarly in pepper *Me3* gene (Djian-Caporalino et al. 2001) and peanut Mae and Mag genes (Garcia et al. 1996). However, among identified genes, *Mi* gene was introduced to tomato cultivars from *S. peruvianum* by using isozyme marker, Aps-1 and DNA markers, Rex-1 (Medina-Filhoand Tanksley 1983; Williamson et al. 1994) and classified as member of the Leucine Zipper, Nucleotide Binding, Leucine-Rich Repeat (NB-LRR) family of plant genes (Milligan et al. 1998). Globally *Mi* gene has been commercially utilized for the development of root knot resistant tomato cultivars. However, constraint associated with this gene such as it become inactive at above 28°C (Holtzmann 1965), was successfully answered by identifying heat stable resistance gene, *Mi*-9 from wild relative *S. arcanum* is localized on the short arm of chromosome 6 (Ammiraju et al. 2003). In India, Reddy et al. (2018) reported H-88-78-1 an advanced tomato breeding line is resistant against *M. incognita.* Further, molecular screening with *Mi* gene linked markers Pmi and Mi2.3 indicated presence of *Mi* gene in H-88-78-1. Similarly, Bhavana et al. (2019) reported immune response of two tomato genotypes such as HAT-310 and HAT-311 and the six crosses with these two resistant sources, such as HAT-311 × Swarna Lalima, HAT-296 × HAT-311, EC-596747 × HAT- 27 311, Swarna Lalima × HAT-310, EC-596743 × HAT-310 and Swarna Lalima × HAT-311 against *M. incognita.* Further, four molecular markers such as JB-1, REX-1, PMi12 and Mi23 were evaluated to for screening nematode resistance in tomato genotypes. In addition, some studies were piloted to screen resistant sources from major vegetables crops against root knot nematodes are presented table 5.

2 Protease inhibitors: Protease (proteolytic enzymes or proteinases) is a group of enzymes hydrolyze peptides bonds of proteins. Proteases were classified into four groups such as cysteine, serine, aspartyl and metallo based on ability to hydrolyze various peptide bonds. Proteases normally required for nematodes to digest protein into amino acids for protein synthesis. Hence, a mechanism to block nematode proteases which are responsible for parasitism through host protease inhibitors are possibly became a most effective strategy to combat phytoparasitic nematodes. In general, all classes of proteinase inhibitors usually occur in plants (Sirohi and Pankaj 2006). The potential of protease inhibitor (serine protease inhibitor, cowpea trypsin inhibitor (*CpTi*)) was first demonstrated in transgenic potato against golden cyst nematode (Hepher and Atkinson, 1992).In India, Papolu *et al.* (2016) found that, transgenic eggplant line expressing a modified rice

Table 5: Root knot nematode resistant/ germplasms in vegetable crops

Vegetable crops	Resistant Variety/Resistant lines	Screening	Root knot nematode species	References
Chilli	CA-960, G-4, Surajmukhi, ZCH-3025, BSBS-172, Pant Chilli-4, LCA-206, Roshni, Brahmpur, CH-1, Byadagi Kaddi	Pot	*M. incognita* Race1	Ravishankar, 2007
Tomato	PT4716A, LE812, Hisar N_1, Hisar N_2, Patriot, SL-120	Pot	*M. incognita*	Indu Rani et al. 2009.
Tomato	PAU Acc.-1, Hisar Lal, Line 8-2-1-2-5, EC531804, Line 1-6-1-4, EC119197, EC631955, EC631956, EC631957, EC631958, PNR-7, IC 90079, IC117012, CR-2-P8-6, EC520075, EC 521079.	Sick plot	*M. incognita*	Kaur et al. 2014
Brinjal	Gachhabaigan, Azadkranti, Kantabaigen, Athagara Local, Kamaghara local, *Solanum indicum* , PBR 129-5, ARU-1, BB1-3, BB 45-C, BB-49, KS-224, Utkal madhuri, BR-112, LB-13, LB-25, LB-28, LB-30, LB-44, LB-5	Pot	*M. incognita*	Nayak et al. 2015
Tomato	LA 2823, LA 3471, H-88-78-1 and Hisar Lalit	Pot	*M. incognita*	Reddy et al. 2018

cystatin (OC-I1D86) gene under the control of the root-specific promoter, TUB-1 exhibited significant inhibition of reproduction in root knot nematode (*M. incognita*). Similarly, Gawade et al. (2017) found significant reduction of root gall formation and egg mass production on tomato roots treated with *Cicer arietinum* proteinase inhibitor (*CaPI*). These studies showed the pathway to design management strategy for the suppression of root knot nematodes infecting vegetable crops by using protease inhibitors.

RNA interference (RNAi)

In recent years, scientific understanding of plant and nematode interactions at molecular level showed the pathway of genetic engineered resistance against important plant parasitic nematodes. In this arena, discovery of RNA interference in the free-living nematode, *Caenorhabditis elegans* (Fire et al. 1998) emerged as one of the new strategies to combat phytonematodes. RNA interference (RNAi) is a sequence specific and homology-dependent gene silencing mechanism in which double stranded RNA (dsRNA) elicits the post-transcriptional silencing of endogenous genes with homologous sequences. The availability of genome sequence data and bioinformatics tools provided an opportunity to design an effective dsRNA expression constructs for silencing key nematode parasitism genes. Yadav et al. (2006) first successfully demonstrated host induced *Integrase* (dsRNA) gene silencing against *M. incognita* in tobacco, after that many studies were proved the success of RNAi strategy to combat root knot nematodes infecting different crops reviewed by Dutta et al. 2015a, Banerjee et al. 2017, including vegetables for (Dutta et al. 2015b; Shivakumar et al. 2017; Banerjee et al. 2018). Koulagi and Sirohi (2015) developed the protocol for developing resistance against *M. incognita* and tomato leaf curl virus by host generated RNAi through co-transformation in tomato which paves way to pyramid the resistance against more than one pathogen. In this view, RNAi strategy began as new approach to combat this hidden nematode pest. However, possibilities are to be explored to develop nematode resistant cultivars in vegetables crops against this nematode pest by using RNAi strategy.

Integrated approach

In India, numerous attempts have been made to develop several strategies for the management of root knot nematodes in vegetable ecosystem. However, no individual approach of nematode control is free from limitations (Sethi and Gaur, 1986), it is difficult to promote any single approach as effective tool for the management of root knot nematodes in intensive vegetables cultivation. Moreover, influence of soil biotic and abiotic factors leads to greater variability in potentiality of nonchemical approaches than chemical control. For example, nematode antagonists are highly influenced by soil temperature, moisture, texture and structure of soil, soil solarization process completely depends on solar radiation to achieve maximum soil temperature. In addition, requirement of large amount of organic amendment make this approach is quite expensive and also occurrence of unpredictable results. Hence, there is great scope for integrated approach by combining two to more approaches which is based on

Table 6: Available integrated approaches against root knot nematode (*M. incognita*) in different vegetable crops under field conditions

S. No.	Integrated approaches	Crops	Percent reduction of final nematode population in soil	Percent increase in yield over control	References
1.	Solarization of nursery bed + resistant tomato variety (Hisar Lalit) transplanted to main field	Tomato	65.3	56.5	Shanti et al. 2005
2.	Three deep summer ploughings at 15 days interval + neem cake at 20 t/ha + seed treatment (6 g/kg seed) + soil application with a talc formulation of fungal biological control agents (*Pochonia chlamydosporia* (2×10^7 cfu/g) at 12 kg/ha and *Trichoderma viride* (2.8×10^6 cfu/g) at 12 kg ha.	Chilli	70.0	117.0	Singh et al. 2012
3.	Combined application of neem cake (1.5 t/ha) before 10 days of transplanting + soil application of 10 kg/ha talc-based formulation of antagonists *Pseudomonas fluorescens* + *Trichoderma harzianum* enriched with farm yard manure 1.5 t/ha.	Brinjal	89.9	69.6	Singh 2013
4.	Seed treatment with *Pseudomonas fluorescens* 1% W.P. (2×10^8 cfu) @ 20g/kg seed + application of 5 tons of FYM enriched with 2.5 kg of each *Paecilomyces lilacinus* (2 x 10^6 cfu/g) and *Pseudomonas fluorescens* (2 x 10^8 cfu/g)	Okra	82.4	71	Singh et al. 2014
5.	Seed treatment with *Bacillus subtilis* IIHR BS-2 at 10 ml kg/seed+ soil application of 2 tons/ ha of vermicompost enriched with 5 litres of *B. subtilis* IIHR BS-2.	Carrot	69.3	28.8	Rao et al. 2017b

local availability of resources and their reliability. The integrated management approach conceptualized based on principal ‘to reduce the nematode inoculum level below the economic threshold level to derive maximum profits out of the management cost incurred with the least disturbance to the ecological balance’. The studies were conducted in different vegetable crops under field condition to evaluate the efficacy of combination of two or more techniques for the effective root knot nematode management is presented in table 6. Thus, the idea of integrated approach provides better possibilities to minimize the incidence of root knot nematodes and contribute for sustainable vegetable production.

Conclusion

Root-knot nematodes (*Meloidogyne* spp.) are one among the key plant parasitic nematode group become a growing concern for vegetable producers. The phase out of fumigant nematicides due to health and environmental hazards, the problem of root knot nematodes still further intensified and become a major stumbling block for successful cultivation of vegetables in open field as well as protected cultivation. Countrywide distribution of root knot nematode species in vegetable ecosystem was causing huge economic losses in vegetable production. Their management becomes more challenging in intensive vegetable cultivation due to its polyphagous nature, high reproductive potential and unique survival mechanism. Disseminate to new areas through unawareness of nematode infestation, limitation in adopting phytosanitary measures in vegetable nurseries are the emerging threat in vegetable cultivation especially under protected cultivation. Therefore, an approach is warranted to provide basic information and awareness to stake holders through academia, scientific publications and extension activities on root knot nematodes menace in vegetable ecosystem. In this context, an attempt was made to comprehend the significance of root knot nematodes in vegetable cultivation and its nematode management approaches such as cultural methods including prevention, sanitation, summer ploughing, nematode suppressive crops, application of organic amendment etc., physical methods like soil solarization, role of biological control such as fungal and bacterial biocontrol agents, genetics-based methods and chemical control with special reference to India. However, each management approach has its own limitations therefore, integrated nematode management (INM) approach involving the combination of two or more suitable approaches by exploiting locally available resources in a holistic manner are necessary to combat the menace of root knot nematodes in vegetable ecosystem. In addition, possibilities are to be explored to use novel biotechnology tools to develop cost-effective and reliable nematode management strategy.

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सब्जियाँ भारतीय आहार का मुख्य एवं सस्ती घटक हैं जो बढ़ती आबादी को पोषण और आजीविका सुरक्षा सुनिश्चित करने में प्रमुख भूमिका निभाती हैं। जबकि, सब्जी उत्पादन अनेकों जैविक घटकों से बाधित होती है, जो भारत की बढ़ती हुई जनसंख्या की माँगों को पूरा करने में एक वृहद् चुनौती है। कई जैविक घटकों में जड़ गांठ सूत्रकृमि के प्रकोप की भयावकता सबसे विनाशकारी है, जो सब्जी उत्पादन के नुकसान में सबसे बड़ा कारण बन रहा है। जड़ गांठ सूत्रकृमि (*मेलोइडोगाइन* प्रजाति) एक परजीवी, चलने वाला जड़ का अन्तः परजीवी है जो आर्थिक रूप से महत्वपूर्ण समूह में से एक है। सब्जी फसलों को सीधा नुकसान पहुँचाने के अलावा जड़ गांठ मिट्टी के अन्दर ज्यादा संक्रमण के स्तर को बढ़ा देता है जिससे रोग की जटिलता ज्यादा बढ़ जाती है। यद्यपि जड़ गांठ सूत्रकृमियों का प्रबंधन मुख्य रूप से सूत्रकृमि नाशक रसायनों के उपयोग से किया जाता है जबकि सूत्रकृमि नाशक के प्रयोग से स्वास्थ्य एवं बाहरी वातावरण का खतरा बढ़ जाता है। इसके अलावा संरक्षित सब्जियों की खेती में एक प्रमुख रूकावट बन गया है। हमारे देश में सब्जियों में जड़ गांठ सूत्रकृमि प्रबंधन हेतु वैकल्पिक दृष्टिकोण जैसे– सस्य क्रियायें, भौतिक, जैविक, रसायनिक एवं आनुवांशिकीय विधियों को अपनाकर खेती करने की अनुशंसा की जाती है।

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